Characterizing the function of a novel beta-1,3-glucanase from hyperthermophile *Fervidibacter sacchari*

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Abstract
Across the bacterial phylum *Armatimonadota*, we find evidence of polysaccharide specialization. Genes annotated as encoding glycoside hydrolases (GHs)—enzymes that degrade polysaccharides by cleaving the glycosidic linkages between sugar residues—are abundant and diverse, with some members of *Armatimonadota* possessing as many as 348 GHs across 104 GH families. However, only six isolates from *Armatimonadota* have been cultivated, and their saccharolytic lifestyles are under-explored despite phylum-wide specialization. Here, we heterologously express and functionally characterize the GH50 enzyme Fsa16295Glu from the hyperthermophile *Fervidibacter sacchari*, the sole isolate of *Armatimonadota* class *Fervidibacteria*. Using the 3,5-dinitrosalicylic acid assay to evaluate polysaccharide catalysis, we show that the recombinant GH possesses activity on agarose, oat beta-glucan, carboxymethyl curdlan, and laminarin, with the latter three being unusual activities for family GH50, which is primarily a family of agarases. Additionally, we demonstrate that Fsa16295Glu possesses a wide temperature range of 60–95 °C (optimal 80 °C), making it the first characterized hyperthermophilic representative of GH50. Finally, we perform an approximate maximum-likelihood analysis using FastTree to phylogenetically compare Fsa16295Glu to all other known GH50 enzymes and discover that Fsa16295Glu is distant from all other characterized GH50s. This, combined with its unusual endoglucanase activity, prompts us to classify Fsa16295Glu as belonging to a new GH50 subfamily, GH50_3. The characterization of Fsa16295Glu represents the first foray into understanding the biochemistry of polysaccharide degradation by *Armatimonadota*.